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TITLE: A Study of Transrectal Tumor Oxygen Measurements in Patients with Clinically Localized Prostate Cancer

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14. ABSTRACT

This study of tumor hypoxia in prostate cancer patients has completed accrual, and has yielded the largest cohort of its type world-wide. It has provided valuable information about the distribution of hypoxia in both malignant and non-malignant regions of the prostate gland, the relationship between hypoxia and other important clinical and surgico-pathologic prognostic factors and the impact of androgen withdrawal on prostate cancer hypoxia. It has also provided a unique platform for investigating intrinsic biologic markers of tumors hypoxia, and the relationship between hypoxia, DNA repair and radioresistance in a truly relevant clinical context. Information about the relationship between hypoxia and patient outcome will follow at a later time once sufficient patient follow-up is achieved.

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Hypoxia, radiotherapy, malignant progression, clinical study

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Table of Contents

1.0 Introduction

Prostate cancer is the most common malignancy among North American men. At diagnosis, most patients have tumors that clinically are confined to the prostate gland. Depending on co-morbidities and individual preference, they are candidates for potentially curative treatment with either radical prostatectomy or radiotherapy. Despite technical advances in both of these treatments, approximately 25% of radically-treated patients will develop progressive disease either locally in the pelvis or at remote sites, most commonly in bone (1). This underscores the importance of better understanding the biologic factors that are responsible for malignant progression, the development of metastases and the failure of currently available treatments.

Hypoxia is a feature of many human malignancies. In general, patients with hypoxic primary tumors at diagnosis are at greater risk of developing progressive disease and dying regardless of whether initial treatment is with surgery or radiotherapy (2). These clinical observations are consistent with hypoxia-mediated changes in DNA repair, genomic instability and abnormal expression of genes that promote both malignant progression and metastasis formation (3). Faulty DNA repair is an important determinant of genetic instability and contributes to chromosomal re-arrangement, oncogene activation and tumor suppressor gene inactivation (4). Repair-deficient cells are more likely acquire a mutator phenotype characterized by greater biologic and clinical aggressiveness. Indeed, pre-clinical data have shown that hypoxia can lead to the selection of aggressive cancer cells with decreased sensitivity to apoptotic and DNA repair pathways and increased genetic instability, angiogenesis, proliferation and metastatic capability (3, 4).

The primary aims of this clinical study were to measure tumor hypoxia in patients with prostate cancer prior to any treatment and evaluate the independent prognostic significance of hypoxia relative to other recognized clinical and surgico-pathologic determinants of long-term tumor control and patient survival. A secondary aim was to determine the relationship between prostate cancer hypoxia and molecular determinants of radioresistance and DNA repair, and the linkages between these molecular markers and patient outcome.

2.0 Body

This report will discuss progress in relation to the Statement of Work as presented in the initial proposal. It is important to recognize that this award (DMAD-01-1-0111) provided direct financial support for Task 1 only – patient accrual, measurement of tumor hypoxia, collection and banking of prostate biopsies and analysis of molecular markers of radioresistance and DNA repair. Task 1 is now complete and has yielded the largest cohort of patients in the world with hypoxia measurements prior to radiotherapy. Tasks 2 and 3 – patient follow-up and analysis of patient results - are ongoing and fully supported by this institution, specifically the Radiation Medicine Program at Princess Margaret Hospital. Patients with clinically-localized prostate cancer need to be followed for several years after completing treatment before tumor control and overall survival can reliably be determined, reflecting the very long natural history of this disease (5). It has been recommended that radiotherapy studies that use PSA response as the endpoint not be reported until a minimum patient follow-up of 2 years is achieved (5). In addition, to avoid artifacts associated with short follow-up, the reported date of control should be at least 2 years less than the median follow-up of patients accrued to the study (6). This implies that a median follow-up of 7 years is required to reliably report 5 year biochemical control rates. The median follow-up of patients in this study who underwent measurements of tumor hypoxia currently is only 3.5 years, with a range of 0.7 to 7.5 years. Therefore, it is not possible at this time to address the fundamental aim of the project - the independent prognostic significance of

prostate cancer hypoxia in patients. This analysis will be done and reported once appropriate patient follow-up is achieved, and will provide unique information about the biology of this disease and the impact of the tumor microenvironment on tumor progression, response to treatment and long-term patient outcome.

2.1 Task 1:

"Patients will be accrued to this study at a uniform rate of 65 per year (52 eligible patients per year allowing for 20% attrition), over the three years from January 2001 to December 2003. Clinical and surgico-pathologic prognostic information will be collected prospectively at the time each patient enters the study. Eppendorf oxygen measurements will be made. A biopsy will be obtained immediately after the oxygen measurements and evaluated for mutations of the p53 tumor suppressor gene and apoptosis. The biopsies will be processed in batches during the accrual period. A portion of each biopsy will be stored for future study of other hypoxia-related genes. Patients will be accrued to the EF-5 component of the study in the second and third years once phase I testing of this agent is complete in Canada."

Patient Accrual and Measurement of Tumor Hypoxia

Accrual to this clinical study began in August 2001 after the protocol was approved by the Human Subjects Research Review Board of the U.S. Army Medical Research and Materiel Command, and our local institutional Research Ethics Board. The projected accrual was 65 patients annually, to achieve a total study population of 195 patients (156 eligible patients after allowing for attrition).

Accrual to the study was slower than expected as outlined in previous annual reports. The study was originally planned to close on July 31, 2004. However, two extensions were required to assure that the accrual target and goals of the study were met. The study was open a total of 5 years and closed to accrual on July 31, 2006.

Year 1 Accrual	37 patients
Year 2 Accrual	65 patients
Year 3 Accrual	33 patients
Year 4 Accrual	30 patients
Year 5 Accrual	18 patients
Total Accrual	186 patients

One patient accrued to the study in year 3 suffered a serious adverse event. He developed acute prostatitis and suffered a myocardial infarction shortly after the hypoxia measurements were performed. However, he recovered without further complications. This was reported to the University Health Network (UHN) Data Safety Monitoring Board, and the UHN Research Ethics Board. It was also immediately reported to the U.S. Army Medical Research and Materiel Command, and highlighted in the year 3 annual report. No change to either the research protocol or consent form was deemed necessary. There were no other serious adverse events.

Overall, the study closed having accrued a total of 186 patients, which is 95% of the initial accrual target. We anticipate that this will be sufficient to determine the independent prognostic significance of hypoxia in these patients once appropriate follow-up is achieved. It is expected that a median follow-up of 5 years will be reached by early 2009, and that the prognostic factor analysis will be done at that time.

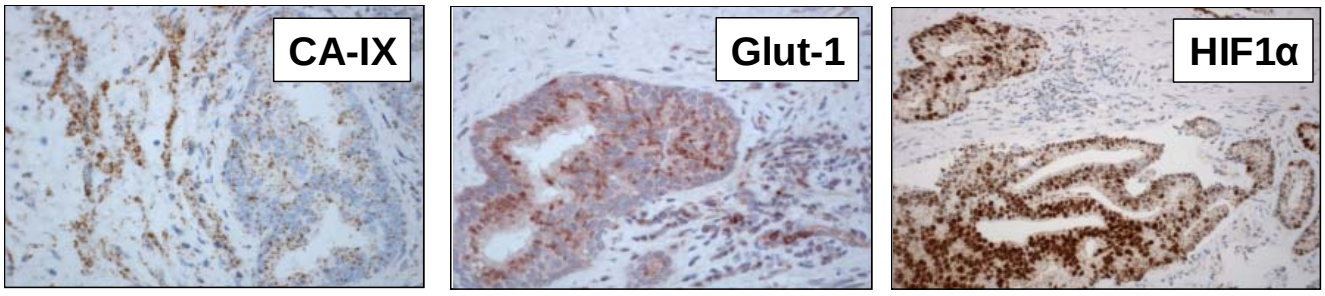
Notwithstanding the fact that information about patient outcome in relation to hypoxia will follow at a later date, several important discoveries have arisen already from this project: We have demonstrated that prostate cancer in patients contains regions of hypoxia of potential biologic and clinical relevance, and that non-malignant regions of the prostate gland in men with prostate cancer may also be hypoxic. These results suggest that hypoxia may be important in both prostate cancer development and in the progression of established disease. We have also demonstrated for the first time in a clinical setting that androgen withdrawal improves prostate hypoxia in some patients. This might in part explain the improved patient outcome that has been observed in many clinical trials of combined treatment with radiotherapy and hormones (7).

Collection and Banking of Prostate Biopsies

A secondary aim of Task 1 was to collect and store biopsies from the same regions of the prostate gland as the hypoxia measurements were made. This aim has also been completed, and has yielded a large tissue bank of frozen prostate biopsies for analysis of hypoxia-related gene and protein expression in relation to radiation response and DNA repair.

Evaluation of Intrinsic Tumor Markers of Hypoxia

A minor component of Task 1 involved the use of the hypoxia marker EF5. As outlined in Section 4.6 of the original protocol, this was to be administered to 30 patients to evaluate the microscopic distribution of oxygen in prostate cancer, and differences in gene expression between oxic and hypoxic regions. This component of the project was amended (approved by the U.S. Army Medical Research and Materiel Command on January 12, 2004) to instead allow the investigation of intrinsic tumor markers of hypoxia (please refer to previous annual reports). These are normal genes and proteins that are up-regulated in the setting of hypoxia. Evaluation of the intrinsic hypoxic markers carbonic anhydrase IX (CA-IX), glucose transporter (GLUT-1) and hypoxia inducible factor-1α (HIF-1α) were completed in collaboration with Dr. Robert Bristow. These markers were selected because each plays an important role in cellular adaptation to hypoxia, and clinical studies in other tumor sites had previously yielded promising results (8-12). Examples of immunohistochemical staining for these three markers in prostate cancer biopsies are shown below:



The table below summarizes the results for 14 patients with prostate cancer. Of the three markers, HIF-1α showed the greatest promise as a marker of hypoxia in prostate cancer. It was up-regulated indicating hypoxia in both malignant and non-malignant regions of the prostate gland. In contrast CA-IX showed non-specific staining and is unlikely to be useful as a marker of prostate cancer hypoxia. A manuscript describing these results has been submitted for publication. Work is ongoing to examine HIF-1α expression in relation to other molecular makers of radioresistance and DNA repair.

Surgical #	Biopsy % Tumour	GLUT-1 (% Bx pos)	GLUT-1 (% CA pos)	HIF-1a (% Bx pos)	HIF-1a (% CA pos)	CA IX location
02-22076 1B	0%	80%	N/A	10%	N/A	stroma
02-21943 1A	60%	30%	30%	65%	100%	stroma
02-21862 1A	60%	70%	50%	50%	100%	stroma
02-20129 1B	30%	100%	100%	20%	80%	stroma
02-9530 1A						stroma
02-17823 1A				0%	0%	stroma
02-10790 1A	100%	40%	40%	60%	60%	stroma
02-17026 1A	30%	30%	0%	5%	20%	stroma
02-17024 1A	95%	100%	100%	10%	10%	stroma
02-16635 1B	100%	100%	100%	85%	85%	stroma
02-15995 1A	60%	100%	100%	90%	100%	stroma
02-17821 1A						stroma
02-14001 1A	80%	95%	100%	80%	100%	stroma
02-12905 1A	95%	100%	100%	100%	100%	stroma

Analysis of Biomarkers of Radioresistance

An addition secondary aim of Task 1 was to use the prostate biopsies to explore the relationship between hypoxia, radioresistance and DNA repair. The initial proposal outlined a limited number of gene and protein expression studies relating to activation of the p53 molecular pathway, which is known to play an important role in radiation response. However, it is now possible to examine multiple genes and gene products relating to this and other pathways simultaneously using tissue micro-arrays (TMA's). TMA's are currently being constructed from the biopsies obtained in this study, again in collaboration with Dr. Robert Bristow. This will provide a more comprehensive description of how hypoxia influences the many molecular pathways involved in radiation response than was envisioned when the proposal was originally written.

2.2 Task 2:

"Patients will be followed for a duration of 3.5 years after completion of accrual in order to realize the required 46 PSA relapses. Patients will be assessed clinically and have PSA measured at regular intervals as part of their routine medical care. The database will be updated on an ongoing basis to reflect current disease and patient status."

All patients accrued to this study are being followed at regular intervals after completion of treatment, as outlined in the protocol. Biochemical, radiographic and clinical recurrences are thoroughly documented. This will continue indefinitely to obtain the most compete information possible about hypoxia as a determinant of patient outcome following treatment with radiotherapy. Regular review of the patient data is performed to assure completeness and accuracy.

2.3 Task 3:

"The comparison of tumor oxygenation to other clinical and surgico-pathologic prognostic factors will be done after completion of accrual (early in year 4). The analysis of the influence of tumor hypoxia on outcome will be done after patients have been followed for an additional 3.5 years (mid year 7)."

Analysis of the relationship between prostate cancer hypoxia and other clinical and surgico-pathologic prognostic factors has been completed (see Reportable Outcomes). No linkages between prostate cancer hypoxia and patient age, prostatic volume, clinical T-category, Gleason score or PSA were identified.

A preliminary analysis of the relationship between hypoxia and patient outcome was performed in 2004, based on the first 150 patients accrued to the previous pilot study and the current study. This was encouraging and supported the hypothesis that hypoxia is an important adverse biologic prognostic factor in prostate cancer. However, the follow-up of patients at the time of this preliminary analysis was <2 years. It has been recommended that radiotherapy studies that use PSA response as the endpoint not be reported until a minimum patient follow-up of 2 years is achieved (5). In addition, to avoid artifacts associated with short follow-up, the reported date of control should be at least 2 years less than the median follow-up of patients accrued to the study (6). This implies that a median follow-up of 7 years is required to reliably report 5 year biochemical control rates, an important endpoint of this study. Therefore, no further analyses of outcome have been undertaken to date. It is anticipated that a median follow-up of 5 years will be reached by early 2009, and that the prognostic factor analysis will be done at that time. This timing is entirely consistent with the initial proposal, in which the analysis of outcome was anticipated to occur 3.5 years after completion of patient accrual.

3.0 Key Research Accomplishments

1. We have measured hypoxia in a total of 186 patients with clinically-localized prostate cancer prior to any treatment. This is the largest cohort of prostate cancer patients world-wide who have undergone assessment of tumor hypoxia. It will yield valuable information about the impact of the tumor microenvironment on tumor progression, response to treatment and long-term patient outcome.
2. We have identified prostate cancer hypoxia of potential biologic and clinical significance in a large proportion of patients with this disease.
3. We have identified hypoxia in non-malignant regions of the prostate gland in men with prostate cancer. This has potential implications for prostate carcinogenesis, since hypoxia is known to induce genetic instability and promote the expression of genes involved in cancer development and malignant progression (3, 4).
4. We have described the spatial distribution and variability of hypoxia readings in the prostate glands of men with prostate cancer.
5. We have shown that prostate cancer hypoxia is independent of patient age, prostatic volume, clinical T-category, Gleason score or PSA.
6. We have demonstrated that androgen withdrawal for a short period of time (1-3 months) reduces prostate cancer hypoxia in some patients. This was done as part of a companion study whereby patients who had hypoxia measurements also participated in a phase III clinical trial of neoadjuvant and concurrent hormonal treatment together with radiotherapy. This suggests that androgen withdrawal may improve the efficiency of oxygen delivery to tumor through an anti-angiogenic mechanism. It is the first time that this effect of androgen withdrawal has been demonstrated in a clinical context and has important implications for future pre-clinical and clinical research.
7. We have assembled a large tissue bank of frozen prostate biopsies, with linkages to clinical parameters, hypoxia measurements and long-term patient follow-up. This will provide a valuable resource for understanding and interpreting the relationship between hypoxia and patient outcome, as well as the relationship between molecular markers of radioresistance and DNA repair and outcome.

8. We have studied intrinsic markers of hypoxia in prostate cancer using the biopsies obtained in this study, and demonstrated HIF-1α to be a potentially important mediator of tumor adaptation to hypoxia.
9. We have generated tissue micro-arrays from the prostate biopsies obtained in this study, which shortly will be used to evaluate the relationship between hypoxia and molecular pathways involved in radiation response and DNA repair.

It is important to note that the most important and potentially most exciting deliverable from this study – the relationship between hypoxia and patient outcome - will follow at a later time once sufficient patient follow-up is achieved.

4.0 Reportable Outcomes

Reportable outcome to date are summarized in the following manuscripts, abstracts and presentations at scientific meetings.

Peer-Reviewed Publications

Parker C, Milosevic M, Toi A, Sweet J, Panzarella T, Bristow R, Catton C, Catton P, Crook J, Gospodarowicz M, Maclean M, Warde P, Hill R. A polarographic electrode study of tumor oxygenation in localized prostate cancer. Int J Radiat Oncol Biol Phys, 58 (Mar), 750-757, 2004.

Milosevic M, Chung P, Parker C, Bristow R, Toi A, Panzarella T, Warde P, Catton C, Menard C, Bayley A, Gospodarowicz M, Hill R. Androgen withdrawal in patients with prostate cancer hypoxia: Implications for disease progression and radiation response. Cancer Res. In press 2007.

Chan N, Milosevic M, Bristow R. Tumor hypoxia, DNA repair and prostate cancer progression: New targets and new therapies. Future Oncology. In press 2007.

Abstracts and Presentations at Scientific Meetings

Milosevic M, Bristow R, Chung P, Panzarella T, Toi A, Hill R. Prostate cancer hypoxia correlates with poor patient outcome following treatment with radiotherapy (Abstract). Int J Radiat Oncol Biol Phys 60(Supp), S236, 2004. Presented at the 2004 Annual Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO).

Milosevic M, Chung P, Bristow R, Toi A, Panzarella T, Hill R. Prostate cancer hypoxia adversely influences outcome following treatment with radiotherapy (Abstract). Radiother Oncology 72 (Supp 1), S8, 2004. Presented at the 2004 Annual Meeting of the Canadian Association of Radiation Oncologists (CARO).

Hypoxia in prostate cancer. 7th Biennial Prostate Cancer Forum. The Prostate Cancer Research Foundation. London, England, June 2006.

Hypoxia in prostate cancer: Prognostic and therapeutic implications. Biomarkers in Prostate Cancer Workshop, Canadian Prostate Cancer Research Initiative. Niagara-on-the Lake, May 2006.

Milosevic M, Chung P, Panzarella T, Toi A, Bristow R, Warde P, Caton C, Menard C, Gospodarowicz M, Hill RP. Androgen deprivation reduces hypoxia in human prostate cancer. Int

J Radiat Oncol Biol Phys 66 (Supp), S12, 2006. Presented at the 2006 Annual Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO).

Prostate cancer hypoxia in patients and the impact of androgen withdrawal: Implications for combined treatment with radiotherapy. Presented at the ASCO/ASTRO/SUO Prostate Cancer Symposium, Orlando, February 2007.

5.0 Conclusions

This study of tumor hypoxia in prostate cancer patients has completed accrual, and has yielded the largest cohort of its type world-wide. It has provided valuable information about the distribution of hypoxia in both malignant and non-malignant regions of the prostate gland, the relationship between hypoxia and other important clinical and surgico-pathologic prognostic factors and the impact of androgen withdrawal on prostate cancer hypoxia. It has also provided a unique platform for investigating intrinsic biologic markers of tumors hypoxia, and the relationship between hypoxia, DNA repair and radioresistance in a truly relevant clinical context. However, the most important and potentially most exciting deliverable from this study – the relationship between hypoxia and patient outcome - will follow at a later time once sufficient patient follow-up is achieved.

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